Dissecting the role of TGFβ signaling in connective tissue precursors for skeletal muscle patterning

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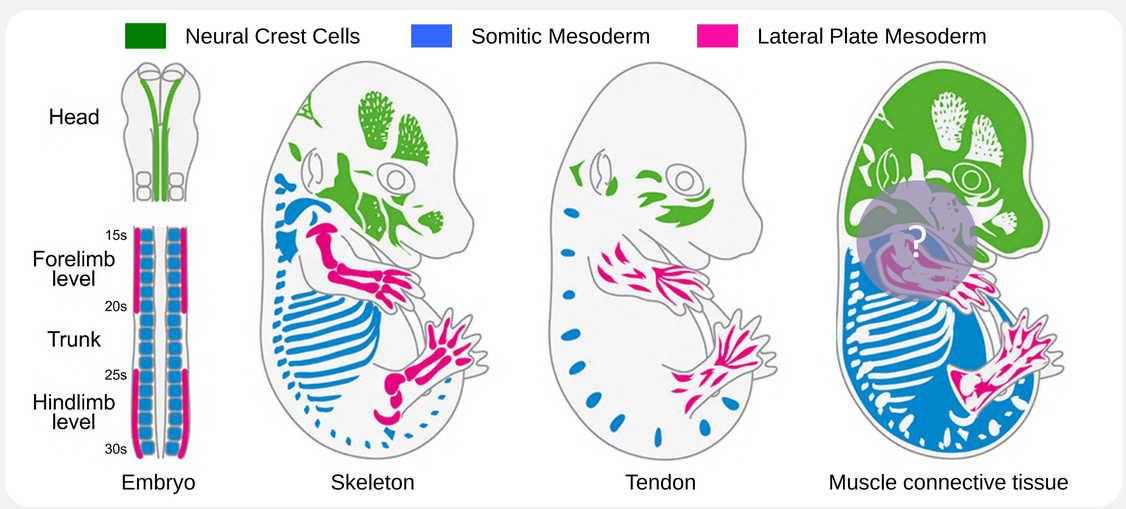
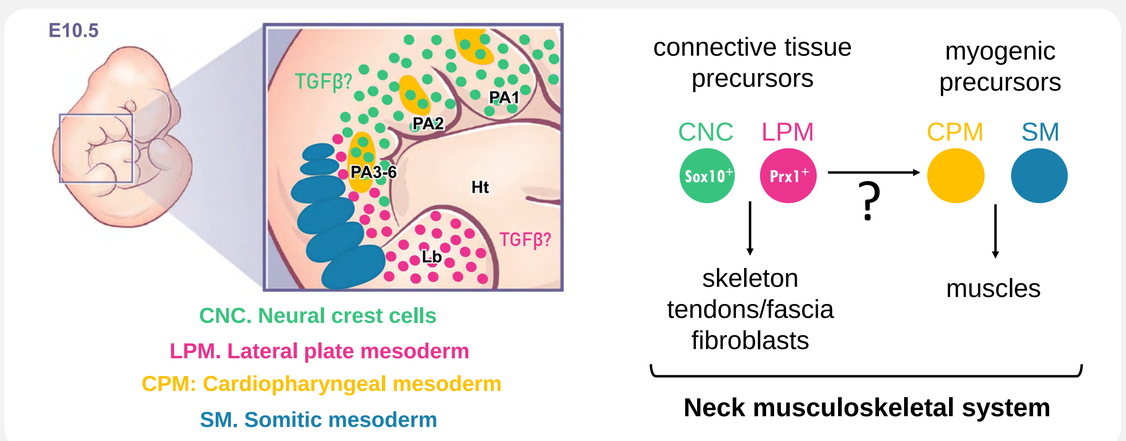
Introduction

While the developmental origins of the vertebrate head, trunk, and limbs are well characterized, the developmental processes that build a functional and integrated musculoskeletal system remain unknown. In specific anatomical contexts, including the head and limbs, previous studies have shown that TGFβ signaling is required in connective tissue precursors for proper skeletal muscle assembly. We aim to define the relative contribution of TGFβ signaling in connective tissue precursors from distinct embryonic sources to musculoskeletal morphogenesis using targeted gene inactivation in the mouse embryo. By investigating how connective tissue progenitors instruct skeletal muscle patterning, this study strives to provide new insights to understand the etiology of some TGFβ-related congenital disorders, such as Loeys-Dietz and Myhre syndromes, for which the musculoskeletal system is affected.

1. Our research objectives

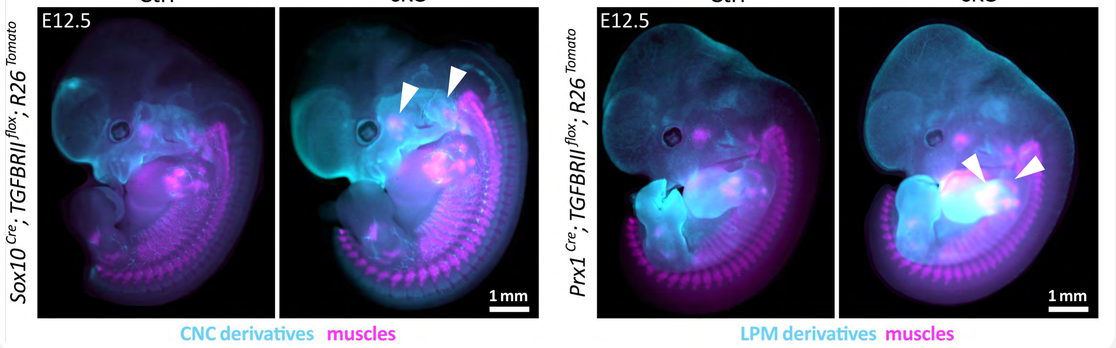


The musculoskeletal systems of the head, trunk and limbs have different embryonic origins. The connective tissue composing the skeleton, tendons and muscle fascia along the axis arises from the neural crest in the head, the somites in the trunk and the lateral plate mesoderm in the limb. The neck constitutes a transition zone at the interface of head, trunk and limb fields. **This project aims to elucidate the role of connective tissue precursors from different embryonic sources in musculoskeletal assembly.** *Figure adapted from Nassari et al. 2017.*



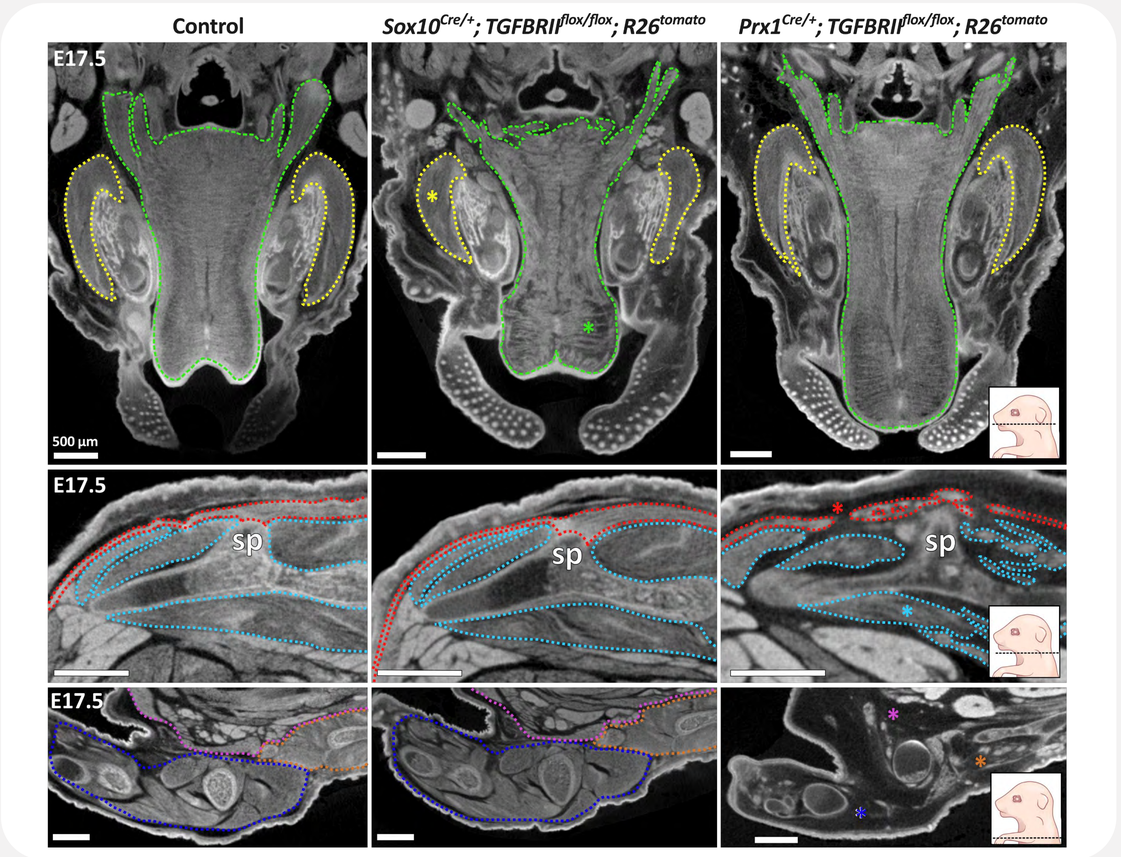
At the head-trunk interface, muscles originate from both CPM and SM populations while the associated connective tissues show intricate contribution from the SM, CNC, CPM and LPM. Connective tissue precursors have emerged as key regulators of skeletal muscle patterning. Some studies suggest that TGFβ signaling plays a role in this process, but the mechanisms by which connective tissue precursors instruct the adjacent developing muscles remain poorly understood. **Using lineage-specific TGFβ-inactivated mice, we aim to identify the cellular and molecular actors of musculoskeletal morphogenesis and the instructive role of connective tissue precursors for musculoskeletal assembly.** *Figure adapted from Heude et al. 2018.*

2. TGFβ signaling in connective tissue precursors is dispensable for initial myogenesis

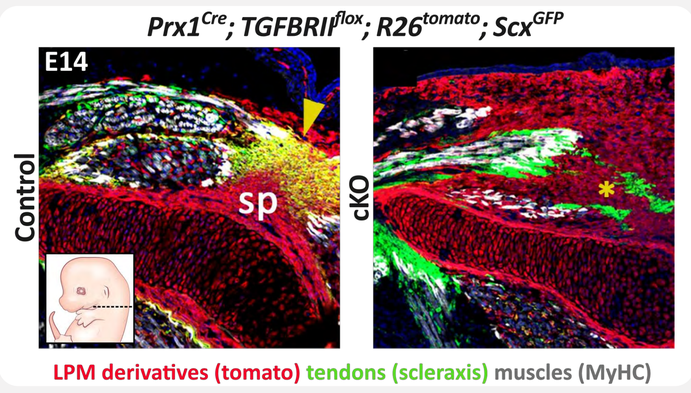


In lineage-specific TGFβ-mutant embryos, muscle precursors are present and initial muscle bundles are correctly formed. Whole-mount IF labelling of Tomato+ CNC/LPM derivatives and of developing muscles (Desmin) in CNC (Sox10-Cre) and LPM (Prx1-Cre) *TGFβRII* mutants and corresponding controls at E12.5. White arrowheads indicate the muscle bundles present in the head of the CNC *TGFβRII* mutant and at the pectoral/limb level in the LPM *TGFβRII* mutant. cKO, conditional knockout.

3. TGFβ signaling in connective tissue precursors from distinct lineages differentially impacts fetal muscle patterning

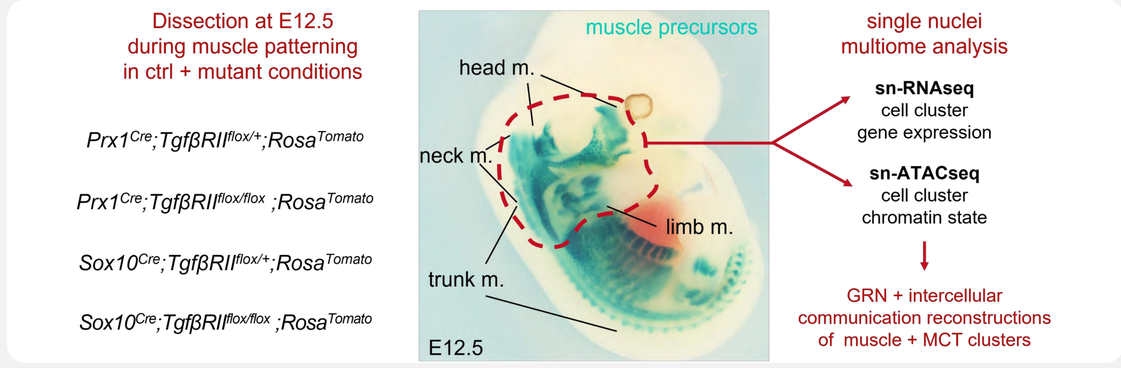


Mutant fetuses reveal a lineage- and region-specific requirement of TGFβ signaling in connective tissue for skeletal muscle patterning. Phenotypic analyses of *TGFβRII* conditional mutants at E17.5 acquired by microtomography. Head muscles are moderately affected in CNC-*TGFβRII* mutants, whereas LPM-*TGFβRII* mutants exhibit severe hypoplasia or agenesis at the pectoral and limb levels. Main muscles groups are delineated: tongue (green), masseter jaw muscle (yellow), scapular musculature (light blue), trapezius (red), limb musculature (dark blue), thoracic muscles (pink), and pectoral musculature (orange). The asterisks indicate when muscles are affected in mutants.



Inactivation of *TGFβRII* in the LPM results in a loss of connection of some neck muscles to the pectoral girdle and loss of LPM tendinous fate suggesting that **TGFβ signaling influences the fate trajectory of LPM-connective tissue cells.**

4. What's next? Identification of TGFβ-dependent Gene Regulatory Networks underlying connective tissue–muscle interactions



Planned experimental set-up for combined snRNA/ATAQ-seq analyses to reconstruct the TGFβ-dependent gene regulatory networks **and identify downstream effectors mediating CT–muscle interactions**.

Conclusion

By defining how connective tissue precursors instruct skeletal muscle patterning in a lineage- and context-dependent manner, this project will provide a new mechanistic framework to understand the basis of connective tissue-muscle communication during musculoskeletal development, relevant to TGFβ-related connective tissue-associated congenital disorders in which the musculoskeletal system is altered.

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